evaluated by dihydroethidium (DHE) and MitoSOX staining. By RT-PCR, CINC-1, IL-1beta, CCL-2 and TNF-alpha gene expression was analyzed in cartilage. IL-8 and Nrf-2 expression were localized in the joint tissue. Finally, CINC-1 expression was evaluated by ELISA.
Results: Joint diameter was significantly increased in OLI-injected knees (0.9 ± 0.1 mm, $n = 8$, $p < 0.05$, vs vehicle-injected joints), similar to LPS-treated knees (2 ± 0.3 mm, $n = 8$, $p < 0.05$, vs vehicle-injected joints). Histological evaluation of synovial tissue by H&E staining revealed that joints treated with mitochondrial inhibitor present greater synovial lining hyperplasia, proliferation of subsynovial tissue and infiltration of a high number of inflammatory cells, while control joints only contained a moderate synovial proliferation and inflammation (3.3 ± 0.1 vs. 2.1 ± 0.2, respectively, $n = 8$, $p < 0.001$, vs vehicle-injected joints). Besides, a higher ROS production was detected in synovial tissue from OLI-injected knees compared to the vehicle-injected counterparts. In this sense, increased expression of the oxidative stress-related transcription factor Nrf-2 was also found in OLI-injected joints. Immunohistochemical studies on IL-8 also showed a greater expression in synovial tissue in OLI-injected joints compared to those from vehicle-injected joints, coinciding with a marked polymorphonuclear cell infiltration. In relation to cartilage, when the loss of matrix in this tissue by safranin O staining was evaluated, no differences were observed. By contrast, when CINC-1 mRNA expression was analyzed in this tissue, a significant increment was detected in OLI-injected joints ($n = 8$, $p < 0.05$ vs. vehicle-injected joint), similar to LPS-treated joints. In addition, we also observed an increment in CINC-1 production by ELISA.
Conclusions: Data seem to support that a loss of mitochondrial function in the joint could participate in rheumatoid pathology through generating an inflammatory and oxidative response in the articular tissue, contributing to the perpetuation of joint injury.

512
M2-MACROPHAGES MODULATE THE CARTILAGE-FORMING CAPACITY OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

S. Sesia, C. Medeiros da Cunha, R. Duhr, E. Padovan, I. Martin, A. Barbero. *Univ.-Hosp. Basel, Basel, Switzerland*

Purpose: Monocytes play a pivotal role in controlling tissue inflammation and repair. They infiltrate the tissues and develop into two main subsets of macrophages in response to local signals: inflammatory (M1-) and tissue-repair (M2-) macrophages (Mφ). Here we investigated whether M1 and M2φ differentially modulate chondrogenesis of mesenchymal stem/stromal cells (MSC)
Methods: Monocytes isolated from buffy-coats were cultured for 5 days with GM-CSF or MCSF to induce respectively M1- or M2φ-polarization. MSC were isolated from the bone marrow of a total of 9 patients and expanded for two passages. MSC or skin fibroblasts (as control cells) and polarized macrophages were cultured in collagen scaffolds up to 3 weeks alone or after being mixed (at the ratio 1:1). In order to determine the reached cell number, selected constructs were generated by combining carboxyfluorescein succinimidyl ester (CFSE) labeled MSC with macrophages. To investigate the role of soluble factors in modulating MSC differentiation, conditioned media harvested from M1φ and M2φ were supplemented during the chondrogenic culture of MSC. Resulting tissues and/or isolate cells were assessed histologically (glycosaminoglycan, GAG), biochemically (GAG contents), cytofluorimetrically (percentage of cells expressing or not CD45, and CFSE) and by RT-PCR (Sox9 and collagen-II)
Results: Coculture of MSC/M2φ in scaffolds resulted in statistically significant 1.9-fold higher GAG content than what would be expected (defined as *chondro-induction*). Chondro-induction was lower (1.3 ± 0.4) and less reproducible when coculture was performed with M1φ and did not occur with skin fibroblasts. GAG contents of constructs generated by solely macrophages were undetectable. Histological analyses of constructs confirmed the biochemical results. In the coculture there was no modulation of the chondrogenic genes. As compared to monocultures, in co-culture MSC and M2φ numbers decreased less markedly (at day 7, MSC were 84% and 42% of the initial number, M2φ were 26% of the initial number and undetectable, respectively for co-cultured and monocultured cells)
Conclusions: We have demonstrated that coculture MSC/Mφ2 results in synergistic cartilage tissue formation, which is not mediated by soluble factors alone. Further studies are envisioned to investigate whether Mφ2 modulate the survival of specific MSC sub-populations. Finally, in vivo studies are necessary to assess the clinical relevance of our findings in the context of cartilage repair.

513
EFFECT OF INFLAMMATION STRESS ON THE HYPERTROPHIC DIFFERENTIATION RELATED GENE EXPRESSION IN CULTURED CHONDROCYTES

H. Inoue, Y. Arai, R. Terauchi, S. Nakagawa, M. Saito, S. Tsuchida, T. Matsuki, S. Ichimaru, A. Inoue, T. Kubo. *Dept. of Orthopaedics, Graduate Sch. of Med. Sci., Kyoto, Japan*

Purpose: Chondrocytes are subject to various in vivo stimulation (hydrostatic pressure stress, heat stress, inflammatory stress, low-oxygen stress, etc.). In recent years, it has been reported that hypertrophic differentiation related factors such as matrix metalloproteinases (MMPs) and NF-kB/hypoxia inducible factor (HIF)-2α signals are vital to the onset and progression of osteoarthritis (OA). In last year's poster, we reported on the effects that hydrostatic pressure stress have on such factors. In chondrocytes which were subject to non-physiological pressure triggering OA, no significant increase in the gene expression of HIF-2α, MMP-3 and MMP-13 was observed. As a result, we concluded that factors other than hydrostatic pressure stress trigger the increase of HIF-2α in OA. In this study, the relationship between inflammatory stimulation and hypertrophic differentiation related factors was analyzed. In addition, heat stimulation and glutamine (Gln) administration were carried out for chondroprotective effect, so as to analyze the changes in hypertrophic differentiation related factors in the chondrocytes receiving inflammatory stimulation.
Methods: This study was conducted according to the regulations regarding animal research of Kyoto Prefectural University of Medicine. Chondrocytes were isolated from male 1.2–1.5 kg Japanese white rabbits. The isolated chondrocytes were cultured as monolayers in 12 well plates during 24hours. Chondrocytes in heat stimulation group were cultured in complete medium including 20 mM of Gln. The culture plates were placed in a circulatory hot water bath set at 41 oC for 30 minutes. After 8 hours incubation at 37oC, chondrocytes were treated with IL-1β. After 24 hours, total RNA was extracted and extracted RNAs were reverse transcribed. And gene expression levels of HIF-2α, NF-kB, MMP-3 and MMP-13 were measured by quantitative real-time PCR.
Results: Due to IL-1β stimulation, all gene expressions increased. In groups which were given heat stimulation prior to IL-1β stimulation, the gene expression of NF-kB and HIF-2α remained unchanged; however, the gene expression of MMP-3 and MMP-13 decreased significantly.
Conclusions: Inflammation is considered to be important to the onset of OA. Likewise in this study, NF-kB/ HIF-2α signal due to inflammatory stimulation, and the gene expression in its downstream, namely MMP-3 and MMP-13, increased. In the past, we clarified that adequate heat stimulation and Gln brings about chondroprotective effects. Also in this study, the increase in gene expression of MMP-3 and MMP-13 resulting from inflammatory stimulation decreased significantly through Gln administration. However, no significant changes were observed in the gene expression of HIF-2α and NF-kB, thus revealing that the chondroprotective effects through heat stimulation does not channel through HIF-2α or NF-kB.

514
GROWTH FACTOR AND FIBRIN SCAFFOLD PROPERTIES ARE INFLUENCED BY THE INCLUSION OF WHITE BLOOD CELLS IN PLATELET-RICH PLASMA

G. Orive, M. Troya, M. Zalduendo, S. Padilla, E. Anitua. *BTI IMASD, Vitoria, Spain*

Purpose: A wide range of studies have shown that platelet-rich plasma (PRP) preparations differ with respect to the inclusion of certain blood components, mainly white blood cells, which may affect the properties of the PRP therapy and host's cellular response. The purpose of this study was to evaluate the morphological, biomechanical and biological responses of leukocyte-rich PRP (L-PRP) versus non-leukocyte PRP preparations both in non-inflammatory and pro-inflammatory cell culture conditions using a commercially available PRP (PRGF: plasma rich in growth factors).
Methods: The following products were generated immediately from the collected blood: plasma rich in growth factors (PRGF) and